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The Cellular Chemistry of ~~SUMMARY~~ Amino Acids

Introduction

The objectives proposed under the above contract embodied three phases:

(1) To identify, as far as possible, the reactions involved in the synthesis of methionine by the mold Neurospora crassa.

(2) To determine the relationship, if any, between methionine synthesis and the synthesis of other amino acids and cellular constituents.

(3) To study the mechanism of the inhibition of the growth of Neurospora by the amino acid canavanine.

As the work progressed, it became evident that objectives (1) and (2) would more than occupy the time of the personnel, and that an attempt to push forward all three objectives simultaneously would substantially reduce the probability of attaining any one in the contract period. It was therefore decided to postpone the investigation of canavanine with the thought in mind that this phase of the investigation might be pursued under an extension of the contract. No advance over what we previously knew was therefore made in the analysis of the canavanine inhibition during the period of this contract.

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The major accomplishments under phases (1) and (2) of the investigation have already been described in the annual report of July 1, 1947. The present report will consist of 2 parts. Part I will briefly summarize the above mentioned annual report, and Part II will describe the work carried on from July 1, 1947 to December 31, 1947.

Part I

The investigation of methionine-requiring mutants of Neurospora established that cysteine is a normal precursor of methionine in the mold. The biological conversion of cysteine to methionine involves a number of chemical steps, of which three were identified in this investigation:

(1) Cysteine + 4-carbon amino acid ⟶ cystathionine

(2) Cystathionine ⟶ Homocysteine

(3) Homocysteine + methyl ⟶ Methionine

It was thus established that cystathionine and homocysteine are intermediates in the conversion of cysteine to methionine. Of special interest was the fact that L-cystathionine was isolated from a mutant strain unable to carry out step (2) of the above scheme. The isolated material was shown to be chemically and biologically indistinguishable from synthetic L-cystathionine. It was thus proven for the first time that cystathionine is a normal intermediate in the biological synthesis of methionine.

It is of interest to note that while methionine synthesis from cysteine does not occur in higher animals, the reverse does take place. It has been shown by other workers (see Annual Report for bibliography) that cysteine production from methionine in mammals involves homocysteine as an intermediate, and it has been suggested that cystathionine is produced in the step from homocysteine to cysteine. In view of our findings, there is now little doubt that cystathionine is formed here. This case provides an interesting illustration of the principle that the most diverse organisms are fundamentally similar in their cellular chemistry.

Part II

A. Tests of other amino acids.

In order to explore further the mechanism of methionine synthesis, a number of amino acids which suggested themselves as possible intermediates in sulfur metabolism were prepared and tested for biological activity. The following substances have been made:

Acetylaminoacrylic acid, CH_2: CHNH ($CCCH_3$) COOH, synthesized according to Bergmann (1). Addition of H_2S followed by splitting-off of the acetyl group would result in cysteine.

Djenkolic acid, CH_2 (SCH_2 $CHNH_2$ COOH)$_2$, prepared according to Armstrong and duVigneaud (2). This substance occurs naturally in djenkol beans. Its obvious relationship to cysteine made it of interest.

"Homodjenkolic" acid, CH_2 (SCH_2 CH_2 $CHNH_2$ COOH)$_2$, a homologue of djenkolic acid, was prepared by a modification of the method of duVigneaud and Patterson (3).

Lanthionine, S(CH_2 $CHNH_2$ COOH)$_2$, prepared by the method of Horn et al. (4). This compound, a homologue of cysthathionine, is obtained from wool.

S-carboxyethyl cysteine, HOOCCH$NH_2$$CH_2$S CH_2 COOH, prepared by the method of Michaelis and Shubert (5), suggested itself as a possible precursor of cysteine.

Tests of these compounds on mutant strains blocked at various stages in methionine synthesis have so far been uniformly negative. Not all of the compounds had been tested on all the strains at the termination of the contract period, however.

B. Investigation of the relationship between methionine and threonine biosyntheses.

In the course of a study of threonine-requiring mutants of Neurospora, it was unexpectedly discovered by H. J. Teas, in collaboration with H. Fling and N. H. Horowitz, that a common precursor exists for both threonine and methionine. It was shown that this precursor is homoserine, CH_2OH CH_2 $CHNH_2$ COOH. This substance turns out to be the 4-carbon amino acid (equation 1.) which condenses with cysteine to give cystathionine. At the same time it is converted by a different series of reactions into threonine.

We have carried out experiments directed toward further elucidation of the methionine-threonine relationship. A number of cross-feeding experiments between methionineless and threonineless mutants were made. It was found that three different methionineless mutants, all lacking the ability to produce cystathionine from cysteine, produce during their growth a substance which promotes the growth of threonine-requiring strains. Tests indicate that the substance is not homoserine, and probably not threonine. The isolation of the active compound is in progress.

Literature cited

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